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For the Patent- and Registration Office

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THERAPEUTIC AGENTS

Field of the invention

The present invention relates to certain novel benzoic acid derivatives, to processes for preparing such compounds, to their utility in treating clinical conditions associated with insulin resistance, to methods for their therapeutic use and to pharmaceutical compositions containing them.

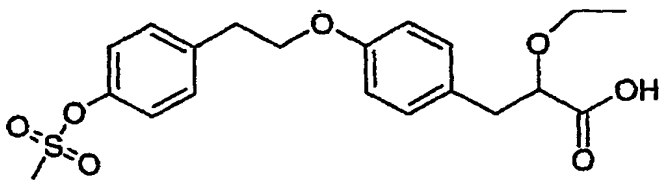
Background of the invention

The Insulin Resistance Syndrome (IRS) including type 2 diabetes mellitus, which refers to a cluster of manifestations including insulin resistance with accompanying hyperinsulinaemia, possible type 2 diabetes mellitus, arterial hypertension, central (visceral) obesity, dyslipidaemia observed as deranged lipoprotein levels typically characterised by elevated VLDL (very low density lipoproteins), small dense LDL particles and reduced HDL (high density lipoprotein) concentrations and reduced fibrinolysis.

Recent epidemiological research has documented that individuals with insulin resistance run a greatly increased risk of cardiovascular morbidity and mortality, notably suffering from myocardial infarction and stroke. In type 2 diabetes mellitus atherosclerosis related conditions cause up to 80% of all deaths.

In clinical medicine there is awareness of the need to increase the insulin sensitivity in IRS suffering patients and thus to correct the dyslipidaemia which is considered to cause the accelerated progress of atherosclerosis. However, currently this is not a universally well defined disease.

The S-enantiomer of the compound of formula C below



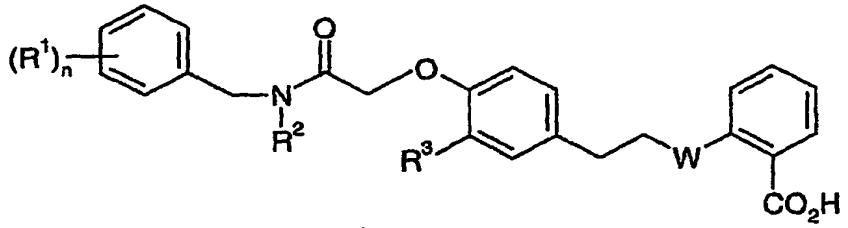
C

2-ethoxy-3-[4-(2-{4-methanesulfonyloxyphenyl}ethoxy)phenyl]propanoic acid, is disclosed in PCT Publication Number WO99/62872. This compound is reported to be a modulator of peroxisome proliferator-activated receptors (PPAR, for a review of the PPARs see T. M.Willson et al , J Med Chem 2000, Vol 43, 527) and has combined PPARα/PPARγ agonist activity (Structure, 2001, Vol 9, 699, P. Cronet et al). This compound is effective in treating conditions associated with insulin resistance.

Surprisingly a series of compounds has now been found which are selective PPARα modulators.

Description of the invention

The present invention provides a compound of formula I



I

wherein n is 0, 1 or 2;

R^1 represents halo, a C_{1-4}alkyl group which is optionally substituted by one or more fluoro, a C_{1-4}alkoxy group which is optionally substituted by one or more fluoro and wherein when n is 2 the substituents R^1 may be the same or different;

R^2 represents an unbranched C_{2-7}alkyl group;

R^3 represents H or OCH_3; and
W represents O or S
and pharmaceutically acceptable salts and prodrugs thereof.

Further values of R^1, R^2, R^3 and W in compounds of Formula I now follow. It will be understood that such values may be used with any of the definitions, claims or embodiments defined hereinbefore or hereinafter.

In a first aspect R^1 is halo, a C_{1-4}alkyl group or a C_{1-4}alkoxy group and n is 0, 1 or 2. Particularly R^1 is fluoro,chloro or trifluoromethyl when n is 1. Particularly R^1 is fluoro when n is 2.

In a second aspect R^2 represents ethyl or hexyl.

In a third aspect R^3 represents H.

In a fourth aspect R^3 represents OMe.

In a fifth aspect W represents O.

In a sixth aspect W represents S.

The term unbranched C_{2-7}alkyl denotes a straight-chain, saturated aliphatic hydrocarbon having from 2 to 7 carbon atoms. Examples of said alkyl include ethyl, n-propyl, n-butyl, n- pentyl, n-hexyl and n- heptyl.

It will be understood by those skilled in the art that the term interrupted as used above means that the oxygen atom is situated within the alkyl chain and is not the terminal atom. The term "prodrug " as used in this specification includes derivatives of the carboxylic acid group which are converted in a mammal, particularly a human, into the carboxylic acid group or a salt or conjugate thereof. It should be understood that, whilst not being bound by theory, it is believed that most of the activity associated with the prodrugs arises from

the activity of the compound of formula I into which the prodrugs are converted. Prodrugs can be prepared by routine methodology well within the capabilities of someone skilled in the art. Various prodrugs of carboxy are known in the art. For examples of such prodrug derivatives, see:

a) Design of Prodrugs, edited by H. Bundgaard, (Elsevier, 1985) and Methods in Enzymology. 42: 309-396, edited by K. Widder, *et al.* (Academic Press, 1985);

b) A Textbook of Drug Design and Development, edited by Krogsgaard-Larsen and H. Bundgaard, Chapter 5 "Design and Application of Prodrugs", by H. Bundgaard p.113-191 (1991);

c) H. Bundgaard, Advanced Drug Delivery Reviews, 8:1-38 (1992);

d) H. Bundgaard, *et al.*, Journal of Pharmaceutical Sciences, 77:285 (1988); and

e) N. Kakeya, *et al.*, Chem Pharm Bull, 32:692 (1984).

The above documents a to e are herein incorporated by reference.

In vivo cleavable esters are just one type of prodrug of the parent molecule.

The compounds of formula I have activity as medicaments, in particular the compounds of formula I are selective agonists of PPARα, that is, their ED_{50} for PPARα is at least four times lower and preferably at least 10 or 50 times lower than their respective ED_{50} for PPARγ wherein the ED_{50}s are measured and calculated as described in the assays later in this document. The compounds of formula I are potent and selective.

Specific compounds of the invention are:

2-[2-(4-{2-[ethyl(2-fluorobenzyl)amino]-2-oxoethoxy}phenyl)ethoxy]benzoic acid;

2-[2-(4-{2-[(2,4-difluorobenzyl)(heptyl)amino]-2-oxoethoxy}-3-methoxyphenyl)-ethoxy]benzoic acid;

2-[2-(4-{2-[(4-chlorobenzyl)(ethyl)amino]-2-oxoethoxy}phenyl)ethoxy]benzoic acid;

2-[2-(4-{2-[ethyl(4-trifluoromethylbenzyl)amino]-2-oxoethoxy}phenyl)ethoxy]benzoic acid ;

2-[2-(4-{2-[ethyl(4-trifluoromethylbenzyl)amino]-2-oxoethoxy}phenyl)ethylthio]benzoic acid ; and

2-[2-(4-{2-[(4-chlorobenzyl)(ethyl)amino]-2-oxoethoxy}phenyl)ethylthio]benzoic acid;

and as well as pharmaceutically acceptable salts, solvates and crystalline forms thereof.

In the present specification the expression "pharmaceutically acceptable salts" is intended to define but is not limited to base salts such as the alkali metal salts, alkaline earth metal salts, ammonium salts, salts with basic amino acids, and salts with organic amines.

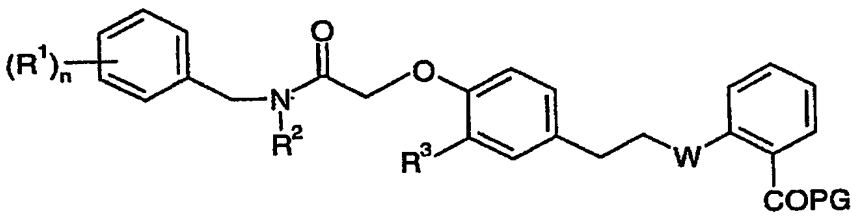
It will also be understood that certain compounds of the present invention may exist in solvated, for example hydrated, as well as unsolvated forms. It is to be understood that the present invention encompasses all such solvated forms. Certain compounds of the present invention may exist as tautomers. It is to be understood that the present invention encompasses all such tautomers.

Throughout the specification and the appended claims, a given chemical formula or name shall encompass all stereo and optical isomers and racemates thereof as well as mixtures in different proportions of the separate enantiomers, where such isomers and enantiomers exist, as well as pharmaceutically acceptable salts thereof and solvates thereof such as for instance hydrates. Isomers may be separated using conventional techniques, e.g. chromatography or fractional crystallisation. The enantiomers may be isolated by separation of racemate for example by fractional crystallisation, resolution or HPLC. The diastereomers may be isolated by separation of isomer mixtures for instance by fractional crystallisation, HPLC or flash chromatography. Alternatively the stereoisomers may be made by chiral synthesis from chiral starting materials under conditions which will not cause racemisation or epimerisation, or by derivatisation, with a chiral reagent. All stereoisomers are included within the scope of the invention.

Methods of preparation

The compounds of the invention may be prepared as outlined below. However, the invention is not limited to these methods, the compounds may also be prepared as described for structurally related compounds in the prior art. The reactions can be carried out according to standard procedures or as described in the experimental section.

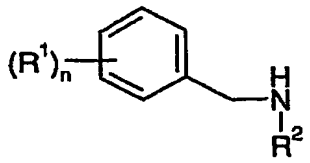
Compounds of formula I may be prepared by reacting a compound of formula II



II

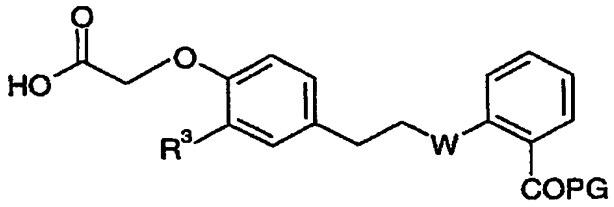
in which R^1, R^2, R^3, Wand n are as previously defined and PG represents a protecting group for a carboxylic hydroxy group as described in the standard text "Protective Groups in Organic Synthesis", 2nd Edition (1991) by Greene and Wuts, with a de-protecting agent. The protecting group may also be a resin, such as Wang resin or 2-chlorotrityl chloride resin. Protecting groups may be removed in accordance to techniques which are well known to those skilled in the art. One such protecting group is where PG represents C_{1-6}alkoxy group or an arylalkoxy group eg benzyl, such that COPG represents an ester. Such esters can be reacted with a hydrolysing agent, for example lithium hydroxide in the presence of a solvent for example a mixture of THF and water or potassium hydroxide in a C_{1-3} alcohol for example methanol , at a temperature in the range of 0-200°C or by microwave radiation to give compounds of formula I.

Compounds of formula II may be prepared by reacting a compound of formula III



III

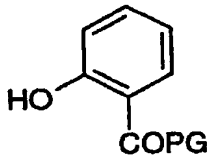
or a salt thereof, for example a hydrochloride salt, in which R^1, R^2 and n are as previously defined with a compound of formula IV



IV

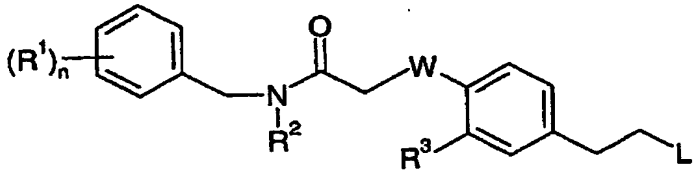
or the acid chloride thereof in which R^3, W and PG are as previously defined in an inert solvent, for example dichloromethane, optionally in the presence of a coupling agent, for example 4-dimethylaminopyridine or 1-ethyl-3-(3-dimethylaminopropyl)carbodiimide hydrochloride, at a temperature in the range of -25°C to 150°C.

Compounds of formula II may also be prepared by reacting a compound of formula V



V

in which PG is as previously defined with a compound of formula VI



VI

in which R^1, R^2, R^3,W and n are as previously defined and L represents a leaving group, for example methylsulphonyloxy or halo, e.g. bromo, optionally in the presence of solvent, for example acetonitrilie, and optionally in the presence of a base, for example potassium carbonate, at a temperature in the range of 0 to 150°C.

Compounds of formula III , IV, V and VI may be prepared by methods described in the Examples or by analogous methods known to those skilled in the art.

Compounds of formula II, III , IV and V are useful intermediates in the preparation of compounds of formula I. Certain of these compounds are believed to be novel. Novel compounds of formula II, or formula III, or formula IV or formula V are herein claimed as a further aspect of the present invention.

The compounds of the invention may be isolated from their reaction mixtures using conventional techniques.

Persons skilled in the art will appreciate that, in order to obtain compounds of the invention in an alternative and in some occasions, more convenient manner, the individual process steps mentioned hereinbefore may be performed in different order, and/or the individual reactions may be performed at different stage in the overall route (i.e. chemical transformations may be performed upon different intermediates to those associated hereinbefore with a particular reaction).

In any of the preceding methods of preparation, where necessary, hydroxy, amino or other reactive groups may be protected using a protecting group, R^P as described in the standard text "Protective groups in Organic Synthesis", 2nd Edition (1991) by Greene and Wuts. The protecting group may also be a resin, such as Wang resin or 2-chlorotrityl chloride resin. The protection and deprotection of functional groups may take place before or after any of the reaction steps described hereinbefore. Protecting groups may be removed in accordance to techniques which are well known to those skilled in the art.

The expression "inert solvent" refers to a solvent which does not react with the starting materials, reagents, intermediates or products in a manner which adversely affects the yield of the desired product.

Pharmaceutical preparations

The compounds of the invention will normally be administered via the oral, parenteral, intravenous, intramuscular, subcutaneous or in other injectable ways, buccal, rectal, vaginal, transdermal and/or nasal route and/or via inhalation, in the form of pharmaceutical preparations comprising the active ingredient either as a free acid, or a pharmaceutical acceptable organic or inorganic base addition salt, in a pharmaceutically acceptable dosage

form. Depending upon the disorder and patient to be treated and the route of administration, the compositions may be administered at varying doses.

Suitable daily doses of the compounds of the invention in therapeutical treatment of humans are about 0.0001-100 mg/kg body weight, preferably 0.001-10 mg/kg body weight.

Oral formulations are preferred particularly tablets or capsules which may be formulated by methods known to those skilled in the art to provide doses of the active compound in the range of 0.5mg to 500mg for example 1 mg, 3 mg, 5 mg, 10 mg, 25mg, 50mg, 100mg and 250mg.

According to a further aspect of the invention there is thus provided a pharmaceutical formulation including any of the compounds of the invention, or pharmaceutically acceptable derivatives thereof, in admixture with pharmaceutically acceptable adjuvants, diluents and/or carriers.

Pharmacological properties

The present compounds of formula (I) are useful for the prophylaxis and/or treatment of clinical conditions associated with inherent or induced reduced sensitivity to insulin (insulin resistance) and associated metabolic disorders. These clinical conditions will include, but will not be limited to, general obesity, abdominal obesity, arterial hypertension, hyperinsulinaemia, hyperglycaemia, type 2 diabetes and the dyslipidaemia characteristically appearing with insulin resistance. This dyslipidaemia, also known as the atherogenic lipoprotein profile, phenotype B, is characterised by moderately elevated non-esterified fatty acids, elevated very low density lipoproteins (VLDL) triglyceride rich particles, high Apo B, low high density lipoproteins (HDL) cholesterol, low apoAI particle levels and the presence of small, dense, low density lipoproteins (LDL) particles. Treatment with the present compounds is expected to lower the cardiovascular morbidity and mortality associated with atherosclerosis due to antidyslipidaemic as well as

antiinflammatory properties. The cardiovascular disease conditions include macro-angiopathies of various internal organs causing myocardial infarction, congestive heart failure, cerebrovascular disease and peripheral arterial insufficiency of the lower extremities. Because of their insulin sensitizing effect the compounds of formula I are also expected to prevent or delay the development of type 2 diabetes from the insulin resistance syndrome and diabetes of pregnancy. Therefore the development of long-term complications associated with chronic hyperglycaemia in diabetes mellitus such as the micro-angiopathies causing renal disease, retinal damage and peripheral vascular disease of the lower limbs are expected to be delayed. Furthermore the compounds may be useful in treatment of various conditions outside the cardiovascular system associated with insulin resistance, like polycystic ovarian syndrome, adipositas, cancer and states of inflammatory disease.

The compounds of the invention may also be combined with other therapeutic agents which are useful in the treatment of disorders associated with the development and progress of atherosclerosis such as hypertension, hyperlipidaemias, dyslipidaemias, diabetes and obesity. In patients with diabetes mellitus the compounds of the invention may also be combined with therapeutic agents used to treat complications related to micro-angiopathies

The compounds of the invention may be used alongside other additional existing therapies for the treatment of type 2 diabetes and its associated complications, these include biguanide drugs, for example metformin, phenformin and buformin, insulin (synthetic insulin analogues, amylin) and oral antihyperglycemics (these are divided into prandial glucose regulators and alpha-glucosidase inhibitors). An example of an alpha-glucosidase inhibitor is acarbose or voglibose or miglitol. An example of a prandial glucose regulator is repaglinide or nateglinide. In addition the combination of the invention may be used in conjunction with another PPAR modulating agent. PPAR modulating agents include but are not limited to thiazolidine-2,4-diones for example troglitazone, ciglitazone, rosiglitazone and pioglitazone. In addition the combination of the invention may be used in conjunction with a sulfonylurea for example: glimepiride, glibenclamide (glyburide), gliclazide, glipizide, gliquidone, chloropropamide, tolbutamide, acetohexamide,

glycopyramide, carbutamide, glibonuride, glisoxepid, glybuthiazole, glibuzole, glyhexamide, glymidine, glypinamide, phenbutamide, tolcylamide and tolazamide. Preferably the sulfonylurea is glimepiride or glibenclamide (glyburide). More preferably the sulfonylurea is glimepiride. Therefore the present invention includes administration of a compound of the present invention in conjunction with one, two or more existing therapies described in this paragraph. The doses of the other existing therapies for the treatment of type 2 diabetes and its associated complications will be those known in the art and approved for use by regulatory bodies for example the FDA and may be found in the Orange Book published by the FDA. Alternatively smaller doses may be used as a result of the benefits derived from the combination.

The present invention also includes a compound of the present invention in combination with a cholesterol-lowering agent. The cholesterol-lowering agents referred to in this application include but are not limited to inhibitors of HMG-CoA reductase (3-hydroxy-3-methylglutaryl coenzyme A reductase). Suitably the HMG-CoA reductase inhibitor is a statin selected from the group consisting of atorvastatin, cerivastatin, fluvastatin, itavastatin, lovastatin, mevastatin, nicostatin, niva-statin, pravastatin and simvastatin, or a pharmaceutically acceptable salt, especially sodium or calcium, or a solvate thereof, or a solvate of such a salt. A particularly preferred statin is, however, a compound with the chemical name (E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl(methylsulfonyl)-amino]-pyrimidin-5-yl](3R,5S)-3,5-dihydroxyhept-6-enoic acid, [also known as (E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[*N*-methyl-*N*-(methylsulfonyl)-amino]pyrimidin-5-yl](3R,5S)-3,5-dihydroxyhept-6-enoic acid] or a pharmaceutically acceptable salt or solvate thereof, or a solvate of such a salt. The compound (E)-7-[4-(4-fluorophenyl)-6-isopropyl-2-[methyl-(methylsulfonyl)-amino]-pyrimidin-5-yl](3R,5S)-3,5-dihydroxyhept-6-enoic acid, and its calcium and sodium salts are disclosed in European Patent Application, Publication No. EP-A-0521471, and in Bioorganic and Medicinal Chemistry, (1997), 5(2), 437-444. This latter statin is now known under its generic name rosuvastatin.

In the present application, the term "cholesterol-lowering agent" also includes chemical modifications of the HMG-CoA reductase inhibitors, such as esters, prodrugs and metabolites, whether active or inactive.

The present invention also includes a compound of the present invention in combination with an inhibitor of the ileal bile acid transport system (IBAT inhibitor) for example those described in WO 93/16055, WO 96/16051, WO 94/18183, WO 94/18184, WO 96/05188 WO 96/08484, WO 97/33882, WO 98/07449, WO 98/03818, WO 99/32478,WO 99/64409, WO 00/01687, WO 00/62810, WO 01/66533, WO 02/32428, EP864582, EP489423, EP549967, EP573848, EP624593, EP624594, EP624595 and EP624596 which are hereby incorporated by reference.

The present invention provides a method of treating or preventing insulin resistance (as defined above) comprising the administration of a compound of formula I to a mammal (particularly a human) in need thereof.

In a further aspect the present invention provides the use of a compound of formula I in the manufacture of a medicament for the treatment of insulin resistance.

Working examples

^{1}H NMR and ^{13}C NMR measurements were performed on a Varian Mercury 300 or Varian UNITY plus 400, 500 or 600 spectrometers, operating at ^{1}H frequencies of 300, 400, 500 and 600 MHz, respectively, and at ^{13}C frequencies of 75, 100, 125 and 150 MHz, respectively. Measurements were made on the delta scale (δ).

Unless otherwise stated, chemical shifts are given in ppm with the solvent as internal standard.

Abbreviations

IRS	insulin resistance syndrome
TLC	thin layer chromatography
HOBT	1-hydroxybenzotriazole-hydrate
DIBAH	diisobutylaluminium hydride
DMSO	dimethyl sulfoxide
EtOAc	ethyl acetate

DMF	*N,N*-dimethylformamide
THF	tetrahydrofuran
HPLC	high performance liquid chromatography
MeCN	acetonitrile
TFA	trifluoroacetic acid
Pd/C	palladium on charcoal
HATU	O-(7-azabenzotriazolyl-1- yl)-N,N,N',N'-tetramethyluronium hexafluorophosphate
DCM	dichloromethane
TBTU	O-(benzotriazol-1- yl)-N,N,N',N'-tetramethyluronium tetrafluoroborate
DIPEA	*N,N*-diisopropylethylamine
DMAP	4-dimethylaminopyridine
Trisamine	Tris(hydroxymethyl)aminomethane

ISOLUTE ® FLASH Si is a silica column suitable for chromatography

Borohydride on polymer support is Borohydride on Amberlite IRA-400 available from Aldrich

LC-MS	liquid chromatography- mass spectroscopy
t	triplet
s	singlet
d	doublet
q	quartet
qvint	quintet
m	multiplet.
br	broad
bs	broad singlet
dm	doublet of multiplet
bt	broad triplet
dd	doublet of doublets

Example 1

a) *Tert*-butyl [4-(2-hydroxyethyl)phenoxy]acetate

A mixture of 4-(2-hydroxyethyl)phenol (3.8ml, 25.834mmol) was dissolved in acetonitrile (25ml), potassium carbonate (7.085g, 51.267mmol) and *tert*-butyl bromoacetate (5.000g, 25.834mmol) was boiled under reflux for 16 hours. The solvent was evaporated under reduced pressure. The residue was dissolved in EtOAc and washed with brine and water, dried with $MgSO_4$ and evaporated under reduced pressure to give the desired product was obtained (6.00g , yield 92.8 %)
^{1}H-NMR (400MHz, $CDCl_3$): 1.52 (s, 9H), 2.98 (t, 2H), 3.46 (t, 2H), 4.92 (s, 2H), 6.89-6.97 (m, 4H)

b) *Tert*-butyl (4-{2-[(methylsulfonyl)oxy]ethyl}phenoxy)acetate

Tert-butyl [4-(2-hydroxyethyl)phenoxy]acetate (6.000g, 23.781mmol) and triethylamine (9.9ml, 71.341mmol) were dissolved in DCM. The mixture was cooled to –10°C and methanesulfonyl chloride (2.8ml, 35.671mmol) was added dropwise to the mixture. The reaction mixture was allowed to reach room temperature and was stirred for 16 hours. The mixture was diluted with DCM. The organic layer was washed with water, brine and 0.3M $KHSO_4$, dried with $MgSO_4$, and evaporated under reduced pressure. Obtained 7.5g of light-yellow crystals (yield 95.5 %).
^{1}H-NMR (400MHz, $CDCl_3$): 1.52 (s, 9H), 2.98 (t, 2H),3.10(s, 3H), 3.46 (t, 2H), 4.92 (s, 2H), 6.89-6.97 (m, 4H)

c) Methyl 2-{2-[4-(2-*tert*-butoxy-2-oxoethoxy)phenyl]ethoxy}benzoate

Methyl salicylate (2.7ml, 21.187mmol) was dissolved in acetonitrile, and potassium carbonate (5.856g, 42.373mmol) was added. The mixture was cooled to –10°C then *tert*-butyl (4-{2-[(methylsulfonyl)oxy]ethyl}phenoxy)acetate was added. The mixture was boiled under reflux for 16 hours, and then the solvent was evaporated under reduced pressure. The residue was dissolved in EtOAc, washed with water and brine, then the

organic layer was dried with $MgSO_4$ and the solvent was removed by evaporation. The crude material was purified by flash chromatography (silica gel 60 0.004-0.063mm) using EtOAc: Toluene 50:50 as the eluant. The fractions which contained the desired product were pooled, and solvent evaporated. This gave 5.0g of pure product (yield 61.1 %).
^{1}H-NMR (400MHz, $CDCl_3$); 1.48 (s, 9H), 3.08 (s, 3H), 3.87 (t, 2H), 4.18 (t, 2H), 4.49 (s, 2H), 6.84 (d, 2H), 6.90-6.98 (m, 2H), 7.20-7.26 (m, 2H), 7.38-7.43 (m,1H), 7.7 (dd, 1H)

d) (4-{2-[2-(methoxycarbonyl)phenoxy]ethyl}phenoxy)acetic acid

Methyl 2-{2-[4-(2-*tert*-butoxy-2-oxoethoxy)phenyl]ethoxy}benzoate (0.400 g, 1.0351mmol) was dissolved in DCM and trifluoracetic acid (0.8ml, 8.281mmol) was added. The mixture was stirred at room temperature for 3h. The solvent was evaporated to give 325 mg of a white powder.
^{1}H-NMR (600MHz, $CDCl_3$); 3.08 (t, 2H), 3.86 (s, 3H), 4.18 (t, 2H), 4.64 (s, 2H), 6.84-6.96 (m, 4H), 7,23 (d, 2H), 7.37-7.42 (m, 1H), 7.75 (dd, 1H)

e) Methyl 2-[2-(4-{2-[ethyl(2-fluorobenzyl)amino]-2- oxoethoxy}phenyl)ethoxy]-benzoate

(4-{2-[2-(Methoxycarbonyl)phenoxy]ethyl}phenoxy)acetic acid (0.200mg, 0.605 mmol) was dissolved in DMF and cooled on an ice-bath. *N*-(2-Fluorobenzyl) ethanamine (0.102 g, 0.666mmol), TBTU (0.214 g, 0.666 mmol) and DIPEA (0.22 ml, 1.271 mmol) was added. The reaction mixture was stirred for 16h at room temperature.EtOAc was added and the organic phase was washed with two portions of 20ml $NaCO_3$ (sat). The organic layer was dried with $MgSO_4$ and the solvent was removed by evaporation. The crude was purified by preparative HPLC (starting with acetonitrile/buffer 60/40 and then increasing the acetonitrile concentration to 100% acetonitrile in 25 min, the buffer was a mixture of acetonitrile/water 10/90 and ammonium acetate (0.1 M), column KR-100-7-C8, 50*500, flow 80ml/min). 145 mg of the desired product was obtained after freeze drying (yield 71.1 %)
^{1}H-NMR (400MHz, CD3CO) (rotamers); 1.08, 1.17 (t, t, 3H), 2.96 (s, 3H), 3.07 (m, 2H), 3.31, 3.36 (m, 2H), 4.21 (m, 2H), 4.85 (s, 2H), 4.56-4.82 (m, 2H), 6.18 (d, 1H), 6.88-7.06 (m, 3H), 7.18 –7.35 (m, 6H), 7.42 (m, 1H), 7.70 (d, 1H)

f) 2-[2-(4-{2-[ethyl(2-fluorobenzyl)amino]-2-oxoethoxy}phenyl)ethoxy]benzoic acid

Methyl 2-[2-(4-{2-[ethyl(2-fluorobenzyl)amino]-2-oxoethoxy}phenyl)ethoxy] benzoate (0.200g, 0.115mmol) was dissolved in 3ml THF in a Smith synthesiser vial and then 1.5 ml water and lithium hydroxide (0.032 g, 1.335mmol) were added to the vial. The vial was capped and put in the microwave oven (Smith synthesiser). The reaction was then heated to 150°C for 6 minutes. According to LC-MS the reaction was complete. The solvent was evaporated. The residue was dissolved in diethyl ether (30 ml) and washed with $NaHCO_3$ (sat) (2x20ml). The basic water layer was acidified to pH 1 with 2M HCl. The water layer was extracted with three portions of 20 ml of DCM which were combined, dried and evaporated to give 160 mg of pure desired product.

^{1}H-NMR (400MHz, CD3CO) (rotamers); 1.07,1.15 (t, t, 3H), 3.10 (m, 2H), 3.30, 3.36 (m, m, 2H), 4.21 (m, 2H), 4.55-4.67 (m, 2H), 4.90 (s, 2H), 6.20 (d,1H), 6.87-7.06 (m, 3H), 7.18-7.35 (m, 6H), 7.40(m, 1H), 7.70 (d, 1H)

Example 2

a) 2-Bromo-*N*-(2,4-difluorobenzyl)-*N*-heptylacetamide

N-(2,4-difluorobenzyl)-*N*-heptylamine(2.004 g, 8.304 mmol) was dissolved in DCM (30 ml). It was then cooled in an ice-bath. Triethylamine (1.092 g, 10.796 mmol) was added and then bromacetyl chloride (1.438 g, 9.135 mmol) was dropped in. The mixture was stirred for 2 hours (ice-bath). It was then washed with water (with additional of 1% hydrochloric acid, pH~3), water and brine, and dried (magnesium sulphate) and evaporated. The crude oil product was dissolved in DCM, then loaded onto a column (ISOLUTE®SI 5g/25 ml) and eluted with more DCM. Oil product 2.412 g was obtained, yield 80%

^{1}H NMR (rotamer, 500 MHz, $CDCl_3$): δ 0.88-0.93 (m, 3H), 1.27-1.34 (m, 8H), 1.52-1.68 (m, 2H), 3.28-3.35 (m, 2H), 3.90-4.15 (m, 2H), 4.61, 4.63 (s, s, 2H), 6.81-6.94 (m, 2H) and 7.15-7.20, 7.34-7.39 (m, 1H).

b) *N*-(2,4-difluorobenzyl)-*N*-heptyl-2-[4-(2-hydroxyethyl)-2-methoxyphenoxy]acetamide

2-Bromo-*N*-(2,4-difluorobenzyl)-*N*-heptylacetamide (135 mg, 0.373 mmol), homovanillyl alcohol (63 mg, 0.373 mmol) and potassium carbonate anhydrous (77 mg, 0.559 mmol) were mixed in acetonitrile (10 ml). The mixture was heated to reflux for 4 hours and then evaporated to dryness. The residue (with additional DCM, 1ml x2) was loaded onto a column (ISOLUTE® SI, 1g/6ml). It was eluted with DCM and then MeOH/DCM (0.5:99.5, then 1:99). The product fractions were combined and evaporated. Oil product 132 mg was obtained yield 79%.

^{1}H NMR (rotamer, 400 MHz, $CDCl_3$): δ 0.82-0.87 (m, 3H), 1.17-1.28 (m, 8H), 1.43-1.68 (m, 2H), 2.75-2.80 (m, 2H), 3.24-3.32 (m, 2H), 3.73-3.84 (m, 5H), 4.58, 4.66 (s, s, 2H), 4.74, 4.76 (s, s, 2H), 6.67-6.86 (m, 5H) and 7.08-7.14, 7.23-7.29 (m, 1H).

c) 2-(4-{2-[(2,4-Difluorobenzyl)(heptyl)amino]-2-oxoethoxy}-3-methoxyphenyl)ethyl methanesulfonate

N-(2,4-difluorobenzyl)-*N*-heptyl-2-[4-(2-hydroxyethyl)-2-methoxyphenoxy]acetamide (A)(132 mg, 0.294 mmol) was dissolved in DCM (10 ml). It was cooled in an ice-bath. Triethylamine (0.05 ml, 0.352 mmol) was added and then methanesulfonyl chloride (37 mg, 0.323 mmol) was dropped in. The cooling-bath was removed after 30 minutes. The mixture was stirred at room temperature overnight. LS-MS showed that ca 50% of A was not reacted. The mixture was cooled in an ice-bath and 0.05 ml of triethylamine was added, followed by 0.025 ml of methanesulfonyl chloride. After addition, the cooling-bath was removed and the mixture was stirred for 5 hours more. It was then washed with water (x2) and brine, dried (magnesium sulphate) and evaporated. Oil product 138 mg was left and used for next step without further purification.

d) Methyl 2-[2-(4-{2-[(2,4-difluorobenzyl)(heptyl)amino]-2-oxoethoxy}-3-methoxyphenyl)ethoxy]benzoate

2-(4-{2-[(2,4-Difluorobenzyl)(heptyl)amino]-2-oxoethoxy}-3-methoxyphenyl)ethyl methanesulfonate (138 mg, 0.262 mmol) was dissolved in acetonitrile (10 ml). 2-

Hydroxybenzoic acid methyl ester (40 mg, 0.262 mmol) was added and then potassium carbonate anhydrous (54 mg, 0.392 mmol) was added. The mixture was heated to reflux overnight and then evaporated to dryness. Water (10 ml) and ethyl acetate (10 ml) were added and the two phases were separated. The organic phase was washed with water and brine, dried (magnesium sulphate) and evaporated. Chromatography of the residue on a column (ISOLUTE® SI, 2 g/ 6 ml) using DCM, MeOH/DCM (1:99) as eluant gave 78 mg the desired product, yield 45% (two steps).

^{1}H NMR (rotamer, 500 MHz, $CDCl_3$): δ 0.87-0.91 (m, 3H), 1.22-1.32 (m, 8H), 1.48-1.63 (m, 2H), 3.09-3.14 (m, 2H), 3.28-3.35 (m, 2H), 3.80, 3.89 (s, s, 3H), 3.89 (s, 3H), 4.21-4.25 (m, 2H), 4.62, 4.71 (s, s, 2H), 4.79, 4.81 (s, s, 2H), 6.77-7.01 (m, 7H), 7.28-7.33 (m, 1H), 7.13-7.18, 7.28-7.33 (m, m, 1H), 7.45 (t, 1H) and 7.81 (d, 1H).

e) 2-[2-(4-{2-[(2,4-Difluorobenzyl)(heptyl)amino]-2-oxoethoxy}-3-methoxyphenyl)ethoxy]benzoic acid

Methyl 2-[2-(4-{2-[(2,4-difluorobenzyl)(heptyl)amino]-2-oxoethoxy}-3-methoxyphenyl)-ethoxy]benzoate (74 mg, 0.127 mmol) dissolved in THF (2 ml) was mixed with lithium hydroxide (6.1 mg, 0.254 mmol) dissolved in water (1 ml). The mixture was irradiated in a microwave oven (Smith Synthesizer) at 150 °C for 8 minutes. LC-MS showed that the reaction was not complete. It was in the oven for additional 10 minutes, LC-MS showed almost no change. 3 mg more of lithium hydroxide was added and thereafter it was in the oven at 150°C for 8 minutes. LC-MS showed it was still the same as before. 3 mg more of lithium hydroxide and 1 ml water was added. The resulting mixture was in the oven at 150 °C for 10 minutes and LC-MS showed the reaction was complete. It was evaporated to remove THF. The residue was acidified with 1% hydrochloric acid, pH~5, and extracted with ethyl acetate (10 ml). The extracts was dried (magnesium sulphate) and evaporated. Chromatography of the residue on a column (ISOLUTE® SI, 1 g/ 6 ml) using DCM and then MeOH/DCM (1:99) as eluant gave 60 mg the desired product, yield 83%.

^{1}H NMR (rotamer, 400 MHz, $CDCl_3$): δ 0.82-0.87 (m, 3H), 1.18-1.28 (m, 8H), 1.43-1.61 (m, 2H), 3.10-3.15(m, 2H), 3.24-3.31 (m, 2H), 3.77, 3.85 (s, s, 3H), 4.39-4.44 (m, 2H),

4.59, 4.66 (s, s, 2H), 4.77, 4.78 (s, s, 2H), 6.72-6.91 (m, 5H), 7.01 (d, 1H), 7.09 (t, 1H), 7.10-7.17, 7.26-7.32 (m, m, 1H), 7.51 (t, 1H) and 8.13 (d, 1H).

^{13}C NMR (rotamers, 75 MHz, $CDCl_3$): δ 14.07, 22.55, 26.79, 27.02, 28.57, 28.93, 31.70, 35.18, 41.30, 41.34, 44.02, 45.89, 46.99, 55.71, 55.82, 68.19, 68.94, 70.66, 103.38(t), 103.88(t), 111.35(d), 111.39(d), 112.32, 112.41, 112.46, 114.76, 117.64, 119.60(dd), 120.07(dd), 120.53, 122.06, 129.50(dd), 130.54, 130.59, 131.55(dd), 133.60, 134.78, 146.26, 146.39, 149.67, 157.10, 161.60(dd), 160.68(dd), 161.97(dd), 162.24(dd), 165.12, 167.75 and 167.93.

The following Examples were prepared in a similar manner:

Example 3

2-[2-(4-{2-[(4-Chlorobenzyl)(ethyl)amino]-2-oxoethoxy}phenyl)ethoxy]benzoic acid.

Example 4

2-[2-(4-{2-[Ethyl(4-trifluoromethylbenzyl)amino]-2-oxoethoxy}phenyl)ethoxy]benzoic acid .

Example 5

2-[2-(4-{2-[Ethyl(4-trifluoromethylbenzyl)amino]-2-oxoethoxy}phenyl)ethylthio]benzoic acid .

Example 6

2-[2-(4-{2-[(4-Chlorobenzyl)(ethyl)amino]-2-oxoethoxy}phenyl)ethylthio]benzoic acid.

Biological activity

Formulations

Compounds were dissolved in DMSO to obtain 16 mM stock solutions. Before assays, stock solutions were further diluted in DMSO and culture media.

GENERAL CHEMICALS AND REAGENTS

Luciferase assay reagent was purchased from Packard, USA. Restriction Enzymes were from Boehringer and Vent polymerase from New England Biolabs.

CELL LINES AND CELL CULTURE CONDITIONS

U2-OS, (Osteogenic sarcoma, Human) was purchased from ATCC, USA. Cells were expanded and refrozen in batches from passage number six. Cells were cultured in Dulbecco's modified Eagle medium (DMEM) with 25 mM glucose, 2 mM glutamine or 4 mM L-alanyl-L-glutamine,10% fetal calf serum, at 5% CO_2. Phosphate buffered saline (PBS) without addition of calcium or magnesium was used. All cell culture reagents were from Gibco (USA) and 96-well cell culture plates were purchased from Wallach.

PLASMID CONSTRUCTS FOR HETEROLOGOUS EXPRESSION

Standard recombinant DNA techniques were carried out as described by Ausubel (7). The Luciferase reporter vector, pGL5UAS (clone consists of five copies of the GAL4 DNA binding sequence, 5'-CGACGGAGTACTGTCCTCCGAGCT-3', cloned into the SacI/XhoI sites of pGL3-Promoter (Promega). The SacI/XhoI fragment carrying the UAS sites was constructed using annealed overlapping oligonucleotides.

Expression vectors used are based upon pSG5 (Stratagene). All vectors contain an EcoRI/NheI fragment encoding the DNA binding domain of GAL4 (encoding amino acid positions 1-145 of database accession number P04386) followed by an in-frame fusion to a fragment encoding the nuclear localisation sequence from T antigen of Polyoma Virus. The nuclear localisation sequence was constructed using annealed overlapping oligonucleotides creating NheI/KpnI sticky ends (5'-CTAGCGCTCCTAGAAGAAACGCAAGGTTGGTAC-3'). The ligand binding domains from human and mouse PPARα and human and mouse PPARγ were PCR amplified as KpnI/BamHI fragments and cloned in frame to the GAL4 DNA binding domain and the nuclear localisation sequence. The sequence of all plasmid constructs used were confirmed by sequencing.

The following expression vectors were used for transient transfections:

vector	encoded PPAR subtype	sequence reference[1]
pSGGALhPPa	human PPARα	S74349, nt 625-1530
pSGGALmPPa	murine PPARα	X57638, nt 668-1573
pSGGALhPPg	human PPARγ	U63415, nt 613-1518
pSGGALmPPg	murine PPARγ	U09138, nt 652-1577

1 refers to nucleotide positions of data base entry used to express the ligand binding domain.

TRANSIENT TRANSFECTIONS

Frozen stocks of cells from passage number six were thawed and expanded to passage number eight before transfections. Confluent cells were trypsinised, washed and pelleted by centrifugation at 270xg for 2 minutes. The cell pellet was resuspended in cold PBS to a cell concentration of about 18 x 10^6 cells/ml. After addition of DNA, the cell suspension was incubated on ice for approximately 5 minutes before electroporation at 230 V, 960 µF in Biorad's Gene Pulser™ in 0.5 ml batches. A total of 50 µg DNA was added to each batch of 0.5 ml cells, including 2.5 µg expression vector, 25 µg reporter vector and 22.5 µg unspecific DNA (pBluescript, Stratagene).

After electroporation, cells were diluted to a concentration of 320'000 cells/ml in DMEM without phenol red, and approximately 25'000 cells/well were seeded in 96-well plates. In order to allow cells to recover, seeded plates were incubated at 37°C for 3-4 hours before addition of test compounds. In assays for PPARα, the cell medium was supplemented with resin-charcoal stripped fetal calf serum (FCS) in order to avoid background activation by fatty acid components of the FCS. The resin-charcoal stripped FCS was produced as

follows; for 500 ml of heat-inactivated FCS, 10 g charcoal and 25 g Bio-Rad Analytical Grade Anion Exchange Resin 200-400 mesh were added, and the solution was kept on a magnetic stirrer at room temperature over night. The following day, the FCS was centrifuged and the stripping procedure was repeated for 4-6 hours. After the second treatment, the FCS was centrifuged and filter sterilised in order to remove remnants of charcoal and resin.

ASSAY PROCEDURE

Stock solutions of compounds in DMSO were diluted in appropriate concentration ranges in master plates. From master plates, compounds were diluted in culture media to obtain test compound solutions for final doses.

After adjustment of the amount of cell medium to 75 µl in each well, 50 µl test compound solution was added. Transiently transfected cells were exposed to compounds for about 24 hours before the luciferase detection assay was performed. For luciferase assays, 100 µl of assay reagent was added manually to each well and plates were left for approximately 20 minutes in order to allow lysis of the cells. After lysis, luciferase activity was measured in a 1420 Multiwell counter, Victor, from Wallach.

Reference compounds

The TZD pioglitazone was used as reference substance for activation of both human and murine PPARγ. 5,8,11,14-Eicosatetrayonic acid (ETYA) was used as reference substance for human PPARα.

Calculations and analysis

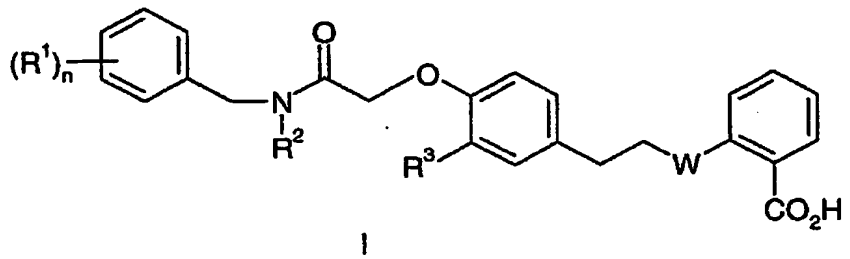
For calculation of ED_{50} values, a concentration-effect curve was established. Values used were derived from the average of two or three independent measurements (after subtraction of the background average value) and were expressed as the percentage of the maximal activation obtained by the reference compound. Values were plotted against the logarithm

of the test compound concentration. ED_{50} values were estimated by linear intercalation between the data points and calculating the concentration required to achieve 50% of the maximal activation obtained by the reference compound.

The compounds of formula I have an ED_{50} of less than 50µmol for PPARα and preferred compounds have an ED_{50} of less than 5µmol.

CLAIMS

1. A compound of formula I



I

wherein n is 0, 1 or 2;

R^1 represents halo, a C_{1-4}alkyl group which is optionally substituted by one or more fluoro, a C_{1-4}alkoxy group which is optionally substituted by one or more fluoro and wherein when n is 2 the substituents R^1 may be the same or different;

R^2 represents an unbranched C_{2-7}alkyl group;

R^3 represents H or OCH_3; and

W represents O or S;

and pharmaceutically acceptable salts and prodrugs thereof.

2. A compound selected from:

2-[2-(4-{2-[ethyl(2-fluorobenzyl)amino]-2-oxoethoxy}phenyl)ethoxy]benzoic acid ;

2-[2-(4-{2-[(2,4-difluorobenzyl)(heptyl)amino]-2-oxoethoxy}-3-methoxyphenyl)-ethoxy]benzoic acid;

2-[2-(4-{2-[(4-chlorobenzyl)(ethyl)amino]-2-oxoethoxy}phenyl)ethoxy]benzoic acid;

2-[2-(4-{2-[ethyl(4-trifluoromethylbenzyl)amino]-2-oxoethoxy}phenyl)ethoxy]benzoic acid ;

2-[2-(4-{2-[ethyl(4-trifluoromethylbenzyl)amino]-2-oxoethoxy}phenyl)ethylthio]benzoic acid ; and

2-[2-(4-{2-[(4-chlorobenzyl)(ethyl)amino]-2-oxoethoxy}phenyl)ethylthio]benzoic acid;

and as well as pharmaceutically acceptable salts, solvates and crystalline forms thereof.

3. A pharmaceutical formulation comprising a compound according to any preceding claim in admixture with pharmaceutically acceptable adjuvants, diluents and/or carriers.

4. A method of treating or preventing insulin resistance comprising the administration of of a compound according to any one of claims 1 to 2 to a mammal in need thereof.

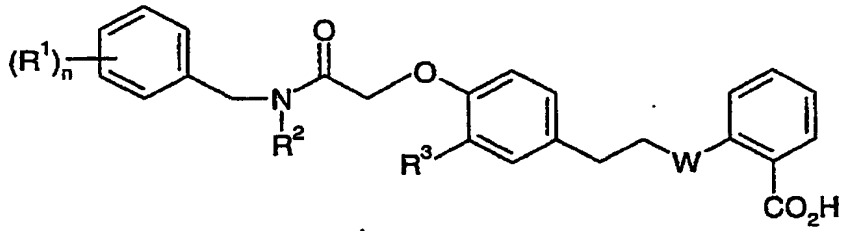
5. The use of a compound according to any one of claims 1 to 2 in the manufacture of a medicament for the treatment of insulin resistance.

6. Processes to prepare acompound of formula I as described herein.

7. Intermediates of formula II, III, IV, V or VI as described herein.

ABSTRACT

The present invention provides a compound of formula I



I

wherein n is 0, 1 or 2;

R^1 represents halo, a C_{1-4}alkyl group which is optionally substituted by one or more fluoro, a C_{1-4}alkoxy group which is optionally substituted by one or more fluoro and wherein when n is 2 the substituents R^1 may be the same or different;

R^2 represents an unbranched C_{2-7}alkyl group;

R^3 represents H or OCH_3; and

W represents O or S

and pharmaceutically acceptable salts and prodrugs thereof , to processes for preparing such compounds, to their utility in treating clinical conditions associated with insulin resistance, to methods for their therapeutic use and to pharmaceutical compositions containing them.